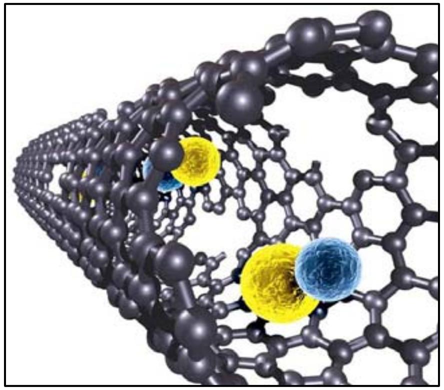
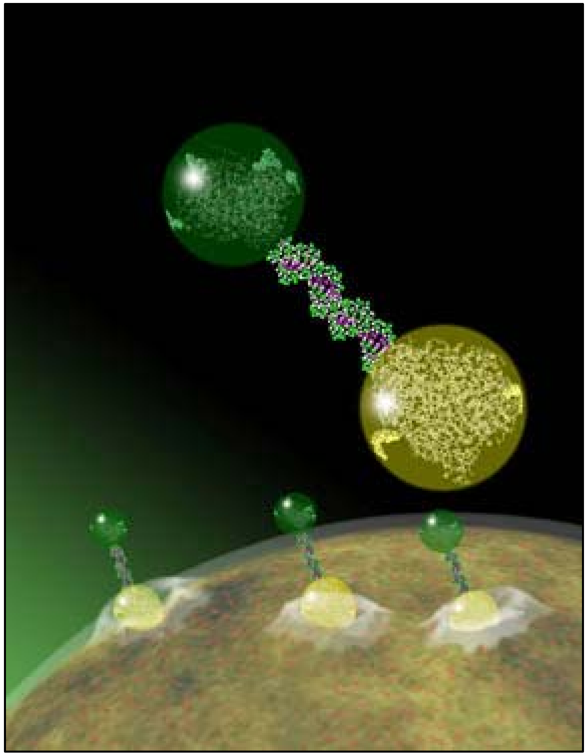
Carbon Nanotubes in Medicine

where we are and where we need to be

Scientific American October 2003

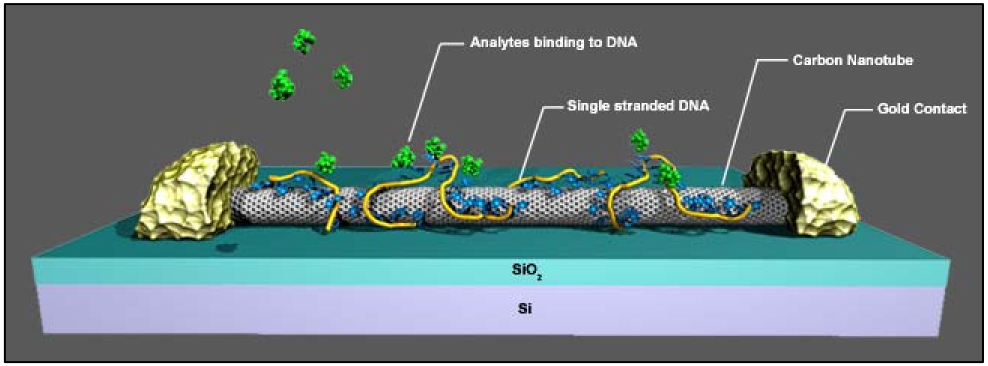


Center for Biological
and Environmental Nanotechnology
Rice University



Michigan Center for Biological Nanotechnology
University of Michigan

Charlie Johnson Group, University of Pennsylvania



Valerie C. Moore
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Carbon Nanotubes in Medicine

where we are and where we need to be

- Carbon Nanotubes in Medicine
  - Motivation
  - Applications
    - MRI Contrast Agents
    - Radical Scavenging Formulations
- Carbon Nanotube Behavior In Vitro and In Vivo
  - Brief Review
  - Highlight Progress in Characterization
  - Examples of Refined Research
- Standards Needs for Biomedical Research

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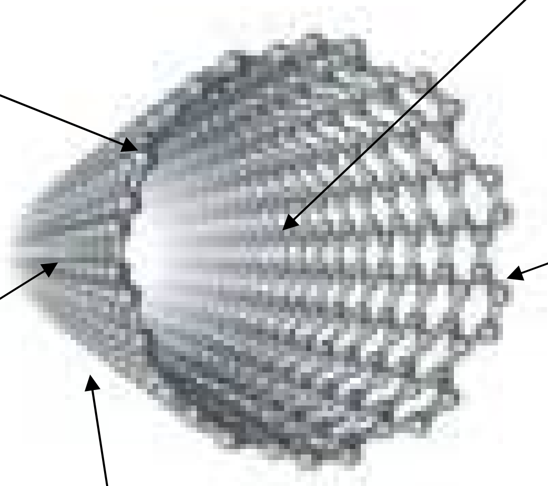
Versatility of Nanotubes

Covalent Sidewall Functionalization

solubility
matrix incorporation
attachment of targets and therapeutics
quenches NIRF, usually

Filling

CT contrast imaging – I_2
MR contrast imaging – Gd^{3+}
cell tracking
carry cargo



Covalent End Functionalization

solubility
matrix incorporation
attachment of targets and therapeutics
allows NIRF with L > 100 nm

Inherent NIRF

contrast imaging
cell tracking
thermal ablation
biomolecule detection

Non-Covalent Sidewall Functionalization

solubility
matrix incorporation
attachment of targets and therapeutics
allows NIR fluorescence and ablation

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Carbon Nanotube Impact on Medicine

- Administration/Implantation
  - Material Engineering
    - strength enhancement
    - electrical
  - Contrast Agents
    - inherent NIR
    - filling
  - Therapeutic Agents
    - ablation
    - drug attachment
    - radical scavenging

- Devices
  - monitor biomolecule levels

- Environmental Health and Safety
  - occupational exposure

Therapeutic Agents

Radical Scavenging Formulations

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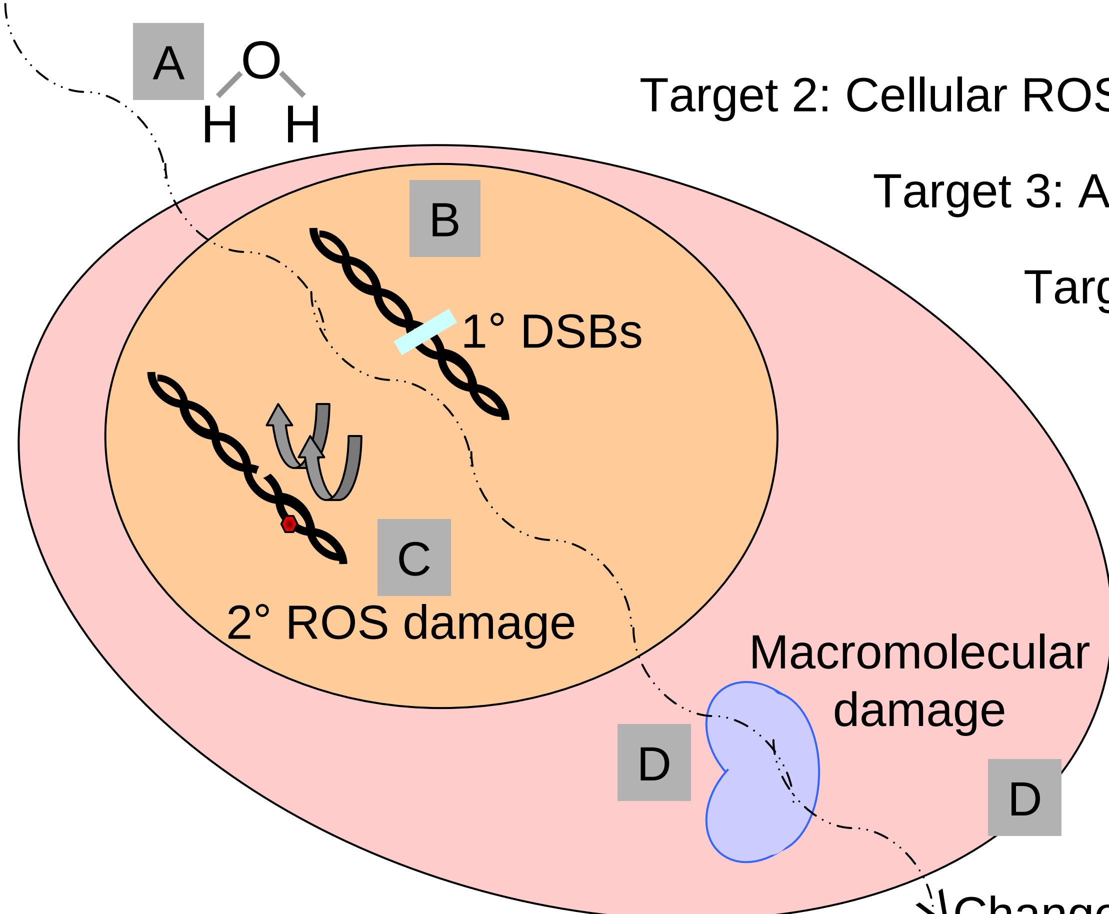
Radiation-Induced Oxidative Stress

Target 1: Extracellular ROS induced by direct radiation (A).

Target 2: Cellular ROS (C,D).

Target 3: Autocrine secondary ROS (E).

Target 4: Paracrine secondary ROS (F).



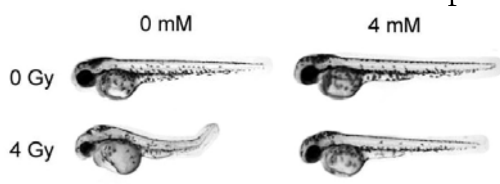
Changes in gene transcription E
Changes in cell cycle

Inflammatory responses F

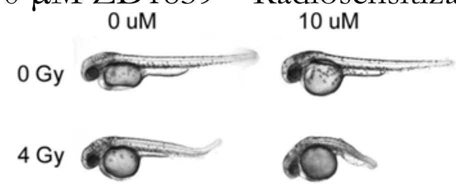
Carbon Nanomaterial Radioprotectors

Zebrafish Morphological Changes with Radiation Exposure

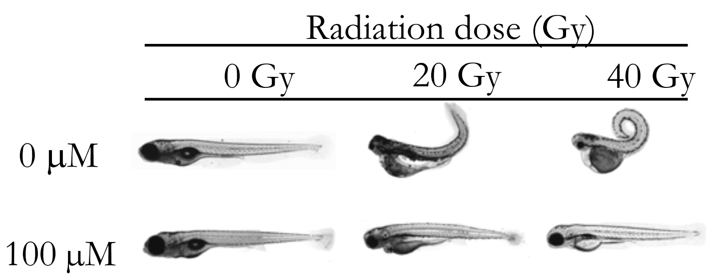
4 mM Amifostine – Radioprotector



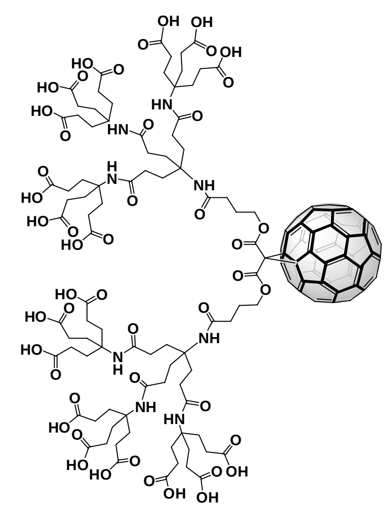
10 μM ZD1839 – Radiosensitization



Efficacy of DF-1 at only 100 μM



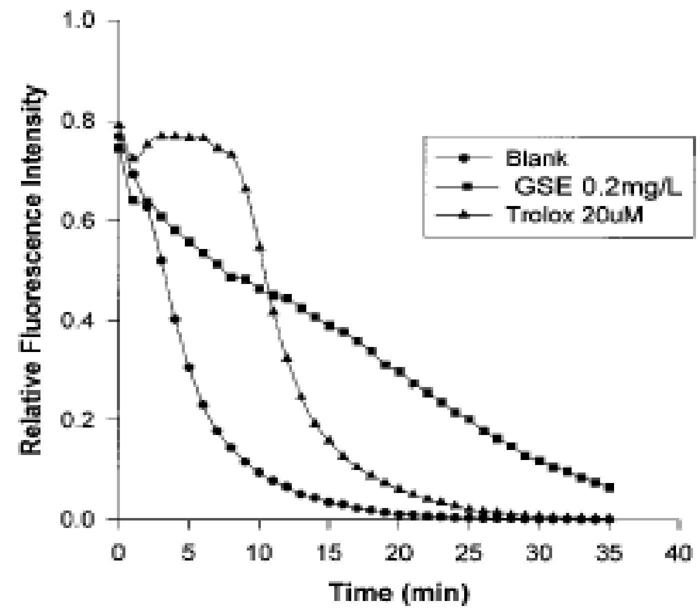
DF-1 was added 3 h prior to IR at 24 hpf
Morphology was assessed either 3 d (20 Gy) or 6 d (40 Gy) post fertilization



B Daroczi, et al. *Clin Cancer Res* 12(23) 7086.

Oxygen Radical Absorbance Capacity

- Radical
  - AAPH thermal initiation
- Radical Scavenger
  - Trolox – assay comparison
  - Amifostine – gold standard
- Indicator
  - Fluorescein

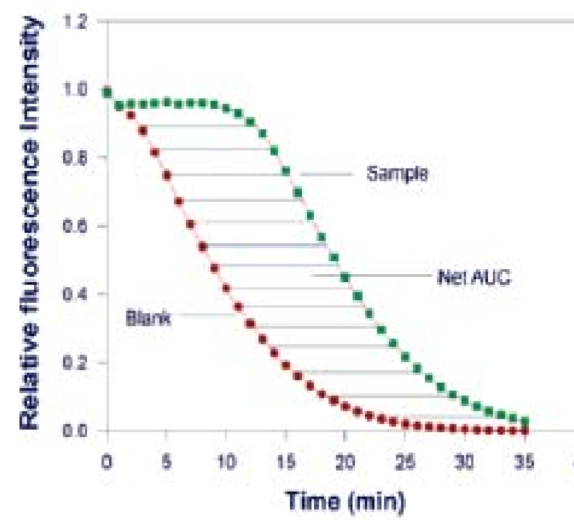


$$R{-}N{=}N{-}R \xrightarrow{O_2} N_2 + 2ROO^{\bullet}\ (14)$$

$$ROO^{\bullet} + \text{probe (fluorescent)} \rightarrow ROOH + \text{oxidized probe (loss of fluorescence)}$$

$$ROO^{\bullet} + AH \rightarrow ROOH + A^{\bullet}$$

$$ROO^{\bullet} + A^{\bullet} \xrightarrow{\text{fast}} ROOA$$

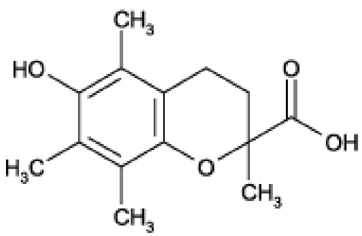


- Calculate Antioxidant Capacity in Trolox Equivalents

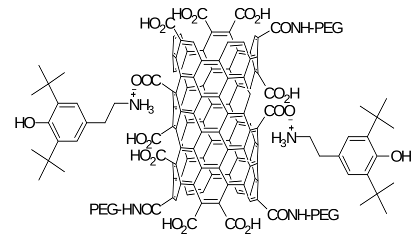
$$\text{relative ORAC value} = [(AUC_{Sample} - AUC_{Blank})/(AUC_{Trolox} - AUC_{Blank})] \times (\text{molarity of Trolox}/\text{molarity of sample})$$

Carbon Nanomaterial Radioprotectors

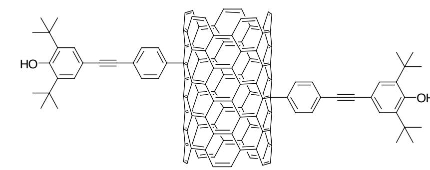
Cell-Free ORAC Assay



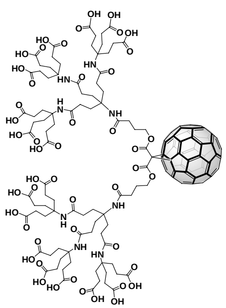
Trolox



Covalent PEG – Ionic BHT



Pluronic Coated – Covalent BHT



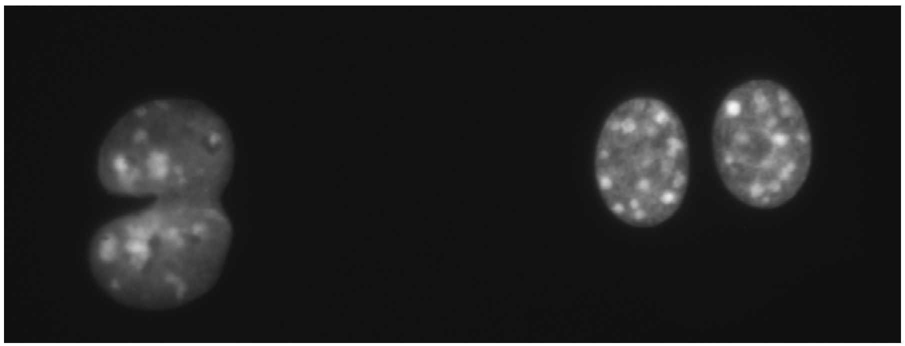
DF-1
dendrite fullerene 1

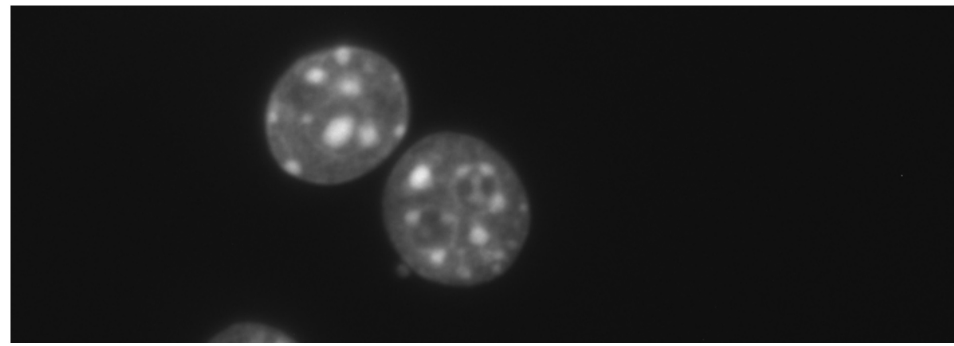
Compound	Trolox Equivalents
DF-1	5
Pluronic Suspended SWCNT Covalent BHT	600
Covalently PEGylated SWCNT Ionic BHT	2400

Work with Rice University, NASA-JSC, and MDACC.

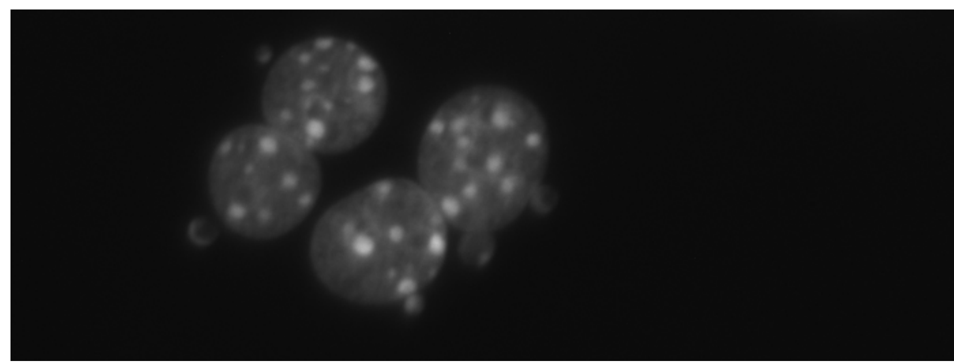
Carbon Nanomaterial Radioprotectors

In Vitro Micronuclei Assay





Radiation	DF-1	+MN	-MN	%MN
0	-	1	521	0.19
0	+	2	567	0.35
2	-	55	688	7.40
2	+	29	668	4.16



Work with Rice University, NASA-JSC, and MDACC.

Carbon Nanotube Behavior In Vitro and In Vivo

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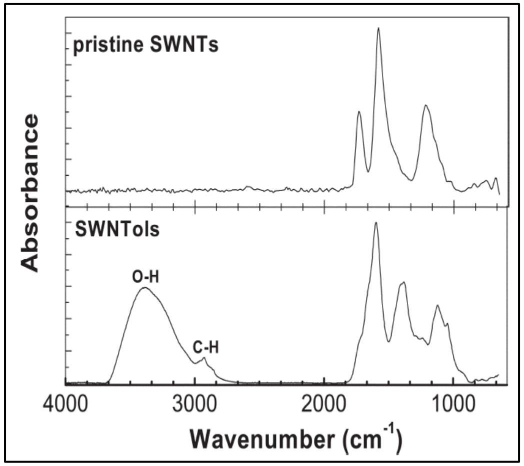
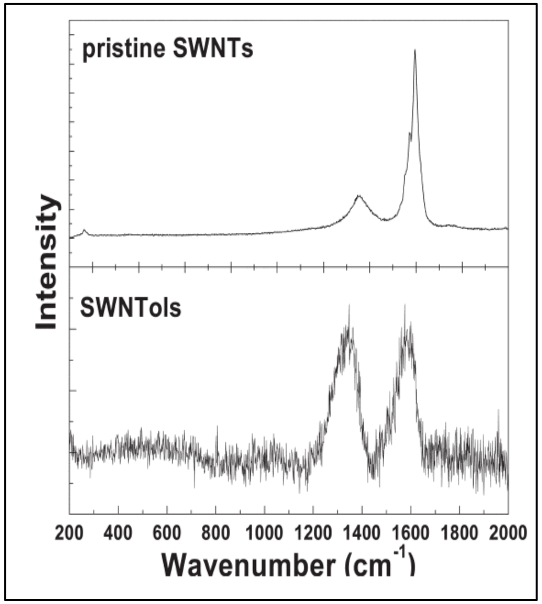
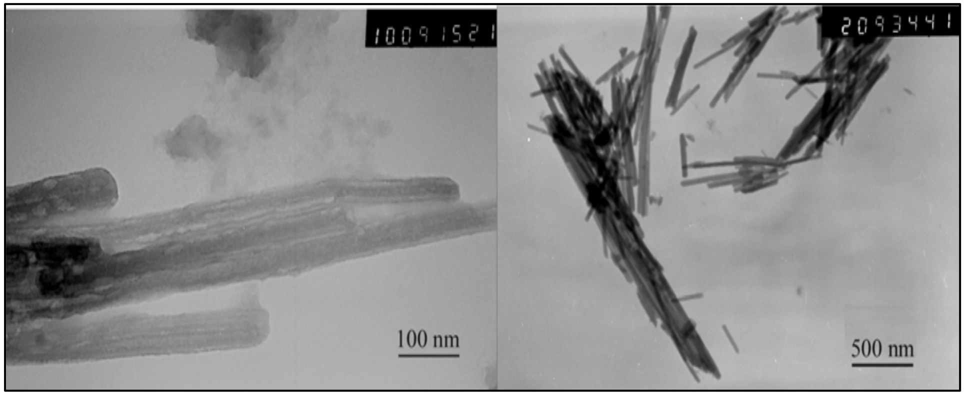
Biodistribution Literature

- 2004 – Wang, et al. investigated the biodistribution of hydroxylated SWCNTs in mice
  - Nanotube Characterization
    - "purity" via Raman and TEM
    - length via laser scattering
      - 280-450 nm
    - concentration via UV-vis absorbance spectroscopy
    - biodistribution via ^{125}I labeling
  - Findings
    - 80% excreted within 11 days
      - 94% urine, 6% feces
    - bone > kidney > stomach





- 2005 – Radomski, et al. investigated nanoparticle-induced vascular thrombosis
  - Nanotube Characterization
    - purchased purified, no independent characterization
  - Findings
    - MCN > SWCNT > MWCNT > standard urban particulate matter induced vascular thrombosis
    - C_{60} did not induce thrombosis



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H Wang, et al. *J. Nanosci. Nanotech.* 4(8), 1019.
A Radomski, et al. *Brit. J. Pharma.* 146, 882.

Biodistribution Literature

- 2006 – Singh, et al. investigated biodistribution of ammonium functionalized SWCNT and MWCNT
  - Nanotube Characterization
    - "purity" from manufacturer
    - diameter from manufacturer
      - ~ 1 nm SWCNT, no distribution given
      - 20-30 nm MWCNT, no distribution given
    - length from manufacturer
      - 300-1000 nm SWCNT, no distribution given
      - 500-2000 nm MWCNT, no distribution given
    - mention in-solution physical characteristics will be different than bulk measurements above
    - biodistribution via ^{111}In labeling
  - Findings
    - kidney > muscle > skin > bone …
    - 3-3.5 hr half life
    - no acute toxicity

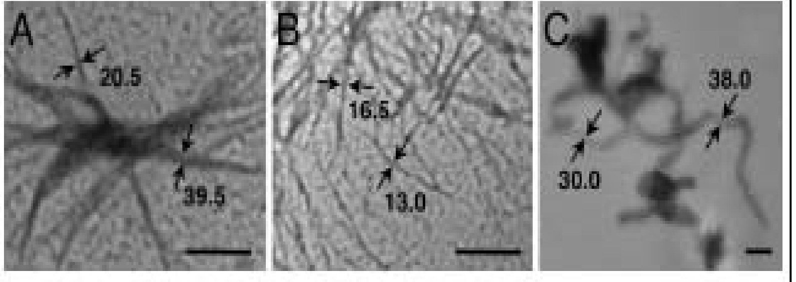
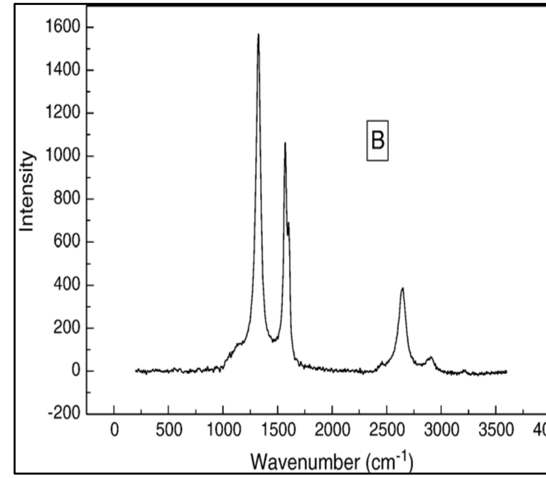


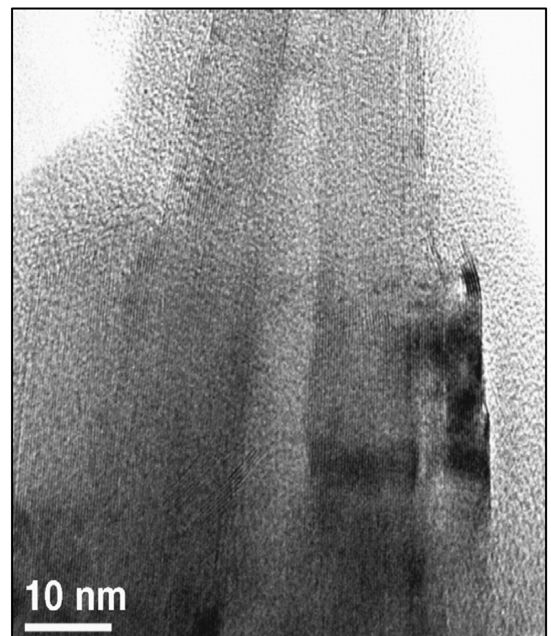
Fig. 1. TEM images of single-walled (*A* and *B*) and multiwalled (*C*) DTPA–CNT. Highly water-soluble and homogeneously dispersed DTPA–CNT were deposited on a TEM grid for observation. (*A* and *B*) DTPA–SWNT form bundles of different length and diameters. Black arrows indicate the dimensions of SWNT bundles, each consisting of 10 and 40 tubes. The thickness of the bundles is in nm. (*C*) DTPA–MWNT were imaged as individual tubes with diameters ≈30–38 nm as indicated by the black arrows. (Scale bars, 200 nm.)

R Singh, et al. *PNAS* 103(9), 3357.

Biodistribution Literature

- 2007 – Guo, et al, investigated biodistribution and clearance of glucosamine functionalized MWCNT
  - Nanotube Characterization
    - measurements made throughout process MWCNTs
      - as-received
      - purified
      - functionalized
    - "purity" via TGA and ICP-MS
      - as-received
        - > 95% MWCNT
        - < 3% amorphous carbon
        - 0.6% Ni
      - Purified
        - > 96% MWCNT
        - < 0.2% Ni
    - diameter and length via TEM
      - 20-40 nm diameter and tens of microns long
    - functionalization confirmation via FTIR
    - biodistribution via ^{99m}Tc labeling
  - Findings
    - 5 hr half-life
    - excretion via urine and feces, roughly 50:50





A Radomski, et al. *Brit. J. Pharma.* 146, 882.

Standards Needed

Standards Needed

- Standard Samples

- Standard Characterization Protocols

1. Composition
   - Catalyst Content
   - Non-NT Carbon
   - Carbon Nanotube Content
2. Aspect Ratio/Length
3. Solution Concentration
4. Surface Functionalization
   - surface charge per unit length
   - degree of functionalization

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